Short Communication

Journal of Biomedical Science

J Biomed Sci 2002;9:721–726
DOI: 10.1159/000067293

Received: May 31, 2002
Accepted: June 14, 2002

A Macrophage-Tropic HIV-1 That Expresses Green Fluorescent Protein and Infects Alveolar and Blood Monocyte-Derived Macrophages

Elizabeth A. Rich[†,a] Jan M. Orenstein[b] Kuan-Teh Jeang[c]

[a]Department of Medicine, Case Western Reserve University, Cleveland, Ohio, [b]Department of Pathology, George Washington University, Washington, D.C., [c]Molecular Virology Section, Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md., USA

Key Words
HIV-1 · Alveolar macrophages · Blood monocytes · Enhanced green fluorescent protein · AIDS · Cigarette smoking

Abstract
We describe the construction of a macrophage-tropic HIV-1 molecular clone, pNLAD8-EGFP, which expresses enhanced green fluorescent protein. We show that NLAD8-EGFP can infect monocyte-derived macrophages as well as alveolar macrophages. NLAD8-EGFP-infected macrophages can be easily and sensitively detected based on the visualization of intracellular green fluorescent protein.

HIV-1 infects CD4+ T lymphocytes as well as monocytes/macrophages. Beyond the circulatory system, tissue macrophages are the predominant substrates for HIV-1 infection in the central nervous system, lungs, lymph nodes, liver and skin/muscle [5, 9, 11, 12, 17, 18, 28, 30, 34, 35]. Macrophages also serve as the major producer of HIV-1 in seropositive individuals with opportunistic infections [23]. Because HIV-1-infected macrophages show minimal cytopathic effects and because macrophages are the initially infected entities after in vivo seroconversion, these long-lived cells may be a primary repository for either actively replicating or latent HIV-1. Indeed, conclusive findings from a recent study in the simian immunodeficiency virus/macaque model system lend credence to the hypothesis that macrophages are the major HIV-1 reservoir in infected humans [14].

Our current understanding of HIV-1 infectivity is that macrophage-tropic (M-tropic) and T cell-tropic (T-tropic) viruses use different cell surface coreceptors [4]. Thus, both M- and T-tropic HIV-1 envelopes bind the CD4 receptor; the former also interacts with the CCR5 coreceptor, while the latter contacts the CXCR4 coreceptor. In vivo, there appears to be an initial negative selection(s) against CXCR4 HIV-1 [37]. Thus, at the early stage of infection, CCR5-M-tropic virus predominates [4]. To better understand virus-macrophage dynamics, it would be useful to have a rapid and sensitive assay of HIV-1 infection in macrophages. To this end, we considered the construction of an M-tropic HIV-1 which expresses enhanced green fluorescent protein (EGFP).

We began with the molecular clone pNLAD8, which is an M-tropic derivative of the T-tropic HIV-1 NL4-3 [2]. pNLAD8 was modified (by Eric Freed) by substituting a *Kpn* I to *Bsm* I fragment from an M-tropic *env* for the counterpart *env* fragment in pNL4-3. As a result of this fragment swap, the chimeric pNLAD8 genome was con-

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1021–7770/02/0096–0721$18.50/0
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Dr. Kuan-Teh Jeang
Building 4, Room 306
9000 Rockville Pike
Bethesda, MD 20892-0460 (USA)
Tel. +1 301 496 6680, Fax +1 301 480 3686, E-Mail kj7e@nih.gov

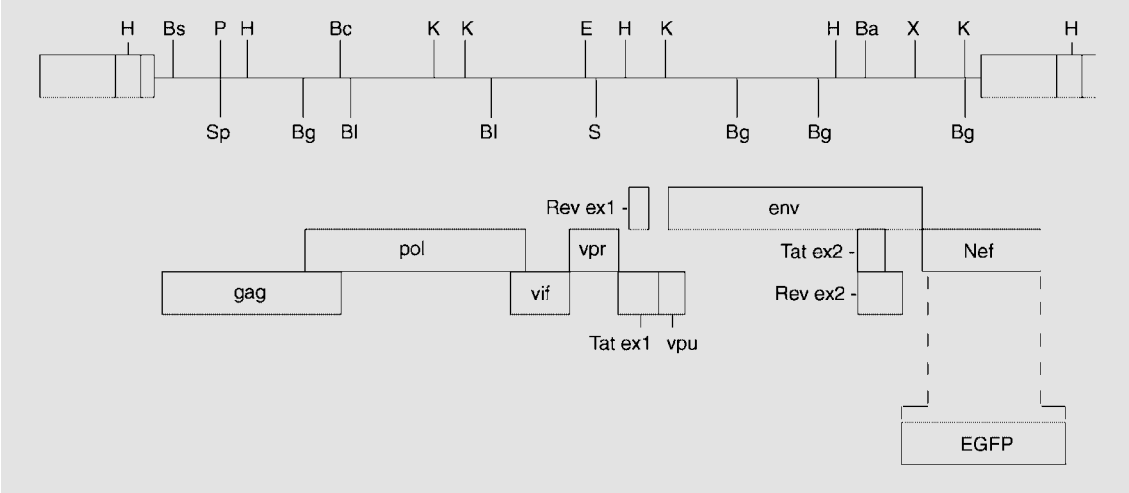

Fig. 1. Schematic representation of pNLAD8-EGFP. pNLAD8 (gift from E. Freed) was constructed by swapping the AD8 envelope sequence contained within a *Kpn* I to *Bsm* I fragment (positions 6347–8053) into pNL4-3. The green fluorescent protein coding sequence from pEGFP-1 (Clontech) was amplified by PCR and inserted in frame into the *Xho* I site of *nef* in pNLAD8 to create pNLAD8-EGFP. pNLAD8-EGFP expresses a functional green fluorescent protein but does not express Nef.

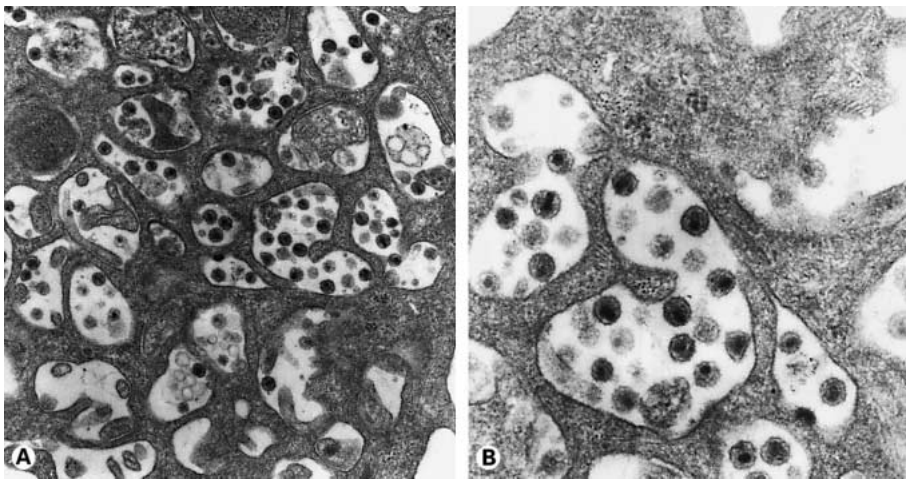

Fig. 2. Electron micrographs of HIV-1 particles in NLAD8-EGFP-infected cultured monocytes. Virus stock was produced by transfection of the molecular clone pNLAD8-EGFP into adherent HeLa cells. Supernatant virus was used to infect culture monocyte-derived macrophages prepared as previously described [1, 29]. On day 10 after infection, cells were fixed with glutaraldehyde and visualized by electron microscopy. A and B show different magnifications.

verted to one fully infectious for cultured macrophages. Previously, we have shown that HIV-1 can efficiently express heterologous open reading frames which are inserted into its *nef* gene [3, 6, 13, 21, 26, 31, 32]. Accordingly, to create an M-tropic HIV-1 which can be rapidly and sensitively detected in cells, we placed an *egfp* cDNA into *nef* of pNLAD8, thus creating the pNLAD8-EGFP molecular clone (fig. 1).

Figure 1 shows a schematic representation of pNLAD8-EGFP. The EGFP open reading frame was amplified by polymerase chain reaction (PCR) from the pEGFP-1 plasmid (Clontech, Palo Alto, Calif., USA) and was inserted in frame into an *Xho* I site downstream of the authentic *nef* AUG. This insertion of EGFP disrupted *nef,* creating the NLAD8-EGFP genome, which is genotypically *egfp(+)/nef(–).*

A priori, it was unclear whether an interrupting insertion of *egfp* into *nef* would affect the infectivity and/or replication competence of NLAD8-EGFP. Elsewhere, studies have suggested that Nef is very important for pro-

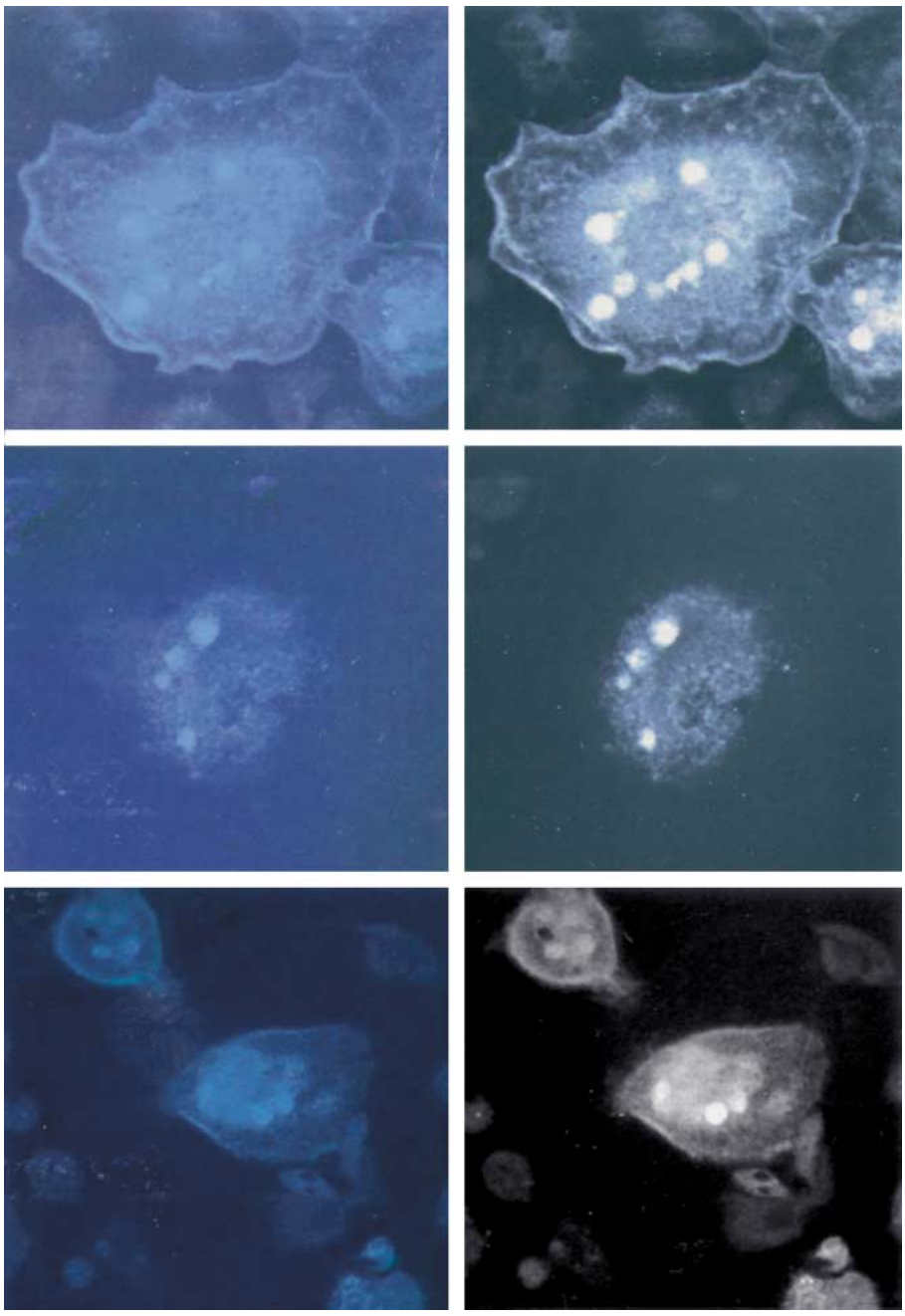

Fig. 3. Cultured monocyte-derived macrophages infected with pNLAD8-EGFP show fluorescent syncytia. Cells were infected with NLAD8-EGFP. On day 20, many multinucleated syncytia were evident. Three examples of fluorescent syncytia visualized by confocal microscopy are shown. Left, blue colored images; right, the same fluorescent images in black and white (bottom).

ductive infection of primary human T lymphocytes and macrophages by HIV-1 [19, 33]. We thus examined this question and sought to verify the cell tropism of NLAD8-EGFP. We investigated infection by NLAD8-EGFP of cultured macrophages derived from primary blood monocytes. NLAD8-EGFP virus was produced by transfecting the molecular plasmid pNLAD8-EGFP into adherent HeLa cells. Forty-eight hours later, a robust amount of reverse transcriptase activity was detected in the cell culture supernatant, consistent with the generation of viral particles. Culture supernatant (approximately 500,000 cpm of reverse transcriptase activity) was collected and used to infect human macrophages prepared as previously described [29]. Ten days after infection, the cells were visualized by electron microscopy. Numerous intracellular HIV-1 particles were clearly seen in the macrophages,

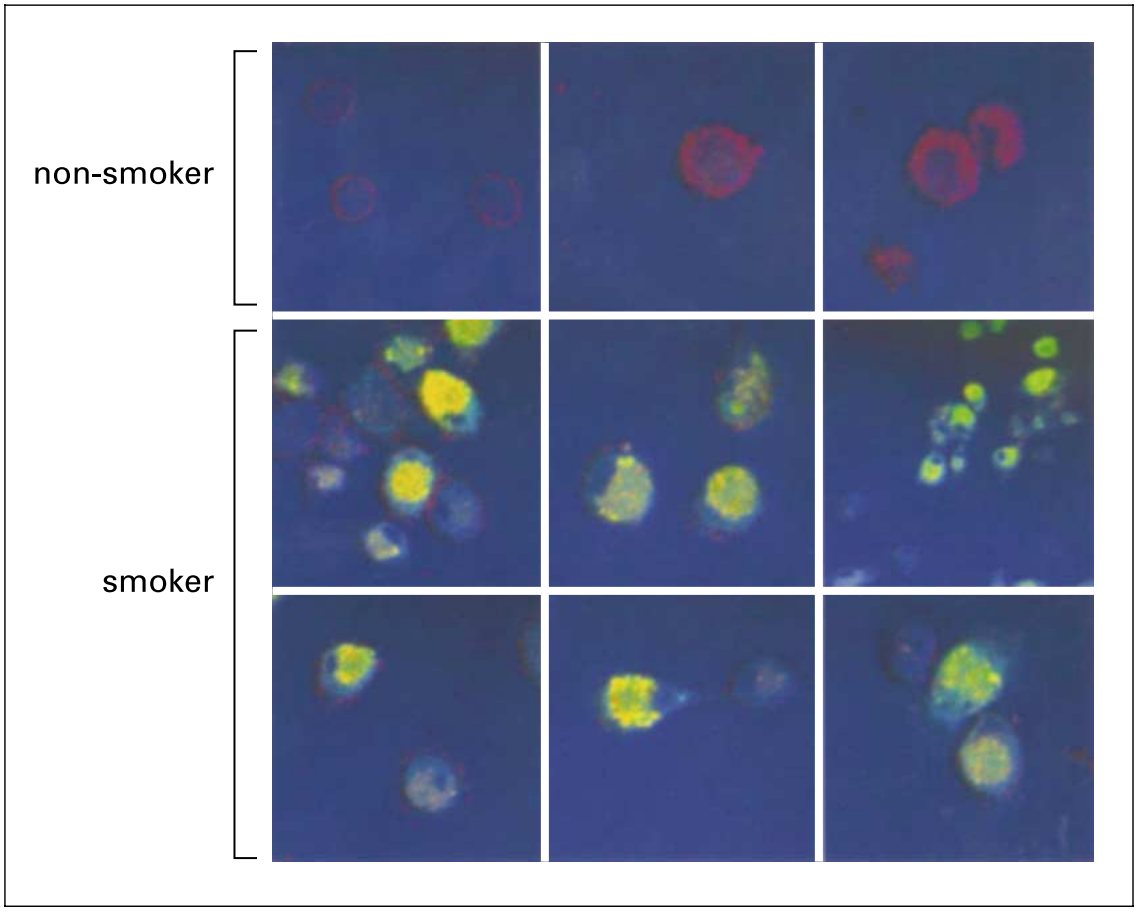

Fig. 4. Preferential infection of AM from healthy cigarette smokers compared to AM from nonsmokers. Preparation and infection of AM from smokers and nonsmokers were performed as previously described [1, 29]. Cells were visualized 20 days after infection with NLAD8-EGFP. Representative fields of view are shown for uninfected control AM (top row, left panel), 'infected' AM from two nonsmokers (top row, middle and right panels) and infected AM from six smokers (middle and bottom rows). Nonfluorescing (and noninfected) AM are evident in the top row (red false color), while EGFP fluorescence (yellow/green false color) is evident in AM from smokers infected ex vivo with NLAD8-EGFP (middle and bottom rows).

confirming the infectivity and cell tropism of the NLAD8-EGFP virus (fig. 2).

HIV-1 infection is minimally cytopathic for macrophages. This cell type-specific property makes it attractive to consider macrophages as a candidate physiological long-term viral reservoir. Consistent with this thought, our NLAD8-EGFP-infected macrophages indeed showed very little cytopathicity even 20 days after virus infection. By this time, formation of multicellular syncytia in the infected cultures was, however, evident. When we illuminated these syncytia by UV and viewed the live cells by confocal microscopy, brightly fluorescent spots consistent with green light emission from NLAD8-EGFP-expressed EGFP protein were easily seen (fig. 3). These fluorescent spots represent facile hallmarks of infection and illustrate the usefulness of NLAD8-EGFP as a sensitive and specific reagent for examining HIV-1 infection of macrophages.

In the lung, alveolar macrophages (AM) are the tissue counterparts of blood monocytes. The lung is a target for HIV infection, and pulmonary complications are frequent causes of morbidity and mortality in AIDS [28]. An ongoing controversy is whether cigarette smoking impacts on pulmonary pathology in HIV-1-infected individuals. Various studies have either refuted [7, 8] or supported [22] a linkage between smoking and AIDS progression. Pre-

viously, Rich and colleagues [1], by measuring peak HIV-1 p24 antigen production, showed that AM from smokers were significantly more productive of ex vivo HIV-1 infection than comparable AM from nonsmokers. Using NLAD8-EGFP, we decided to revisit this question, employing AM from two nonsmokers and six smokers. All eight AM samples were infected ex vivo in parallel with 500,000 cpm of NLAD8-EGFP supernatant. Twenty days after infection, all cultures were visualized for green fluorescent protein. Interestingly, neither the AM from the two nonsmokers nor the control uninfected AM showed EGFP fluorescence (fig. 4, top row). By contrast, all six AM samples from smokers fluoresced intensely, indicating successful infection by NLAD8-EGFP (fig. 4, middle and bottom rows). Pending a larger and better controlled study of infectivity, these results from our limited sampling size of eight would seem to support the interpretation that AM from smokers are more highly activated and infectible by HIV-1 than similar AM from nonsmokers.

Over the past two decades, we have begun to understand the various parameters which govern HIV-1 expression in cells [15, 16]. It has also become increasingly clear that the behavior of HIV-1 in macrophages may not be identical to its behavior in T lymphocytes [10, 20, 25, 27, 38, 39]. Previously, a T-tropic HIV-1 that expresses EGFP, pR7-GFP, was constructed and examined for infectivity [24]. Here, we describe an M-tropic counterpart, pNLAD8-EGFP, which provides a complementing reagent that should permit rapid parallel comparisons/contrasts between HIV-1 infection of T lymphocytes and macrophages. The NLAD8-EGFP virus described here has also been shown recently to capably infect human dendritic cells [36].

Acknowledgements

Part of this study was performed by Elizabeth Rich while she was on sabbatical in the laboratory of K.-T.J. We mourn Elizabeth's untimely passing, and we dedicate this paper to her memory. We thank Ben Berkhout and Christine Neuveut for critical readings and Lan Lin for assistance in the preparation of the manuscript.

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